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QTL ANALYSIS FOR RICE SEEDLINGS UNDER NITROGEN DEFICIENCY USING CHROMOSOMAL SEGMENT SUBSTITUTION LINES

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Abstract

A population of Chromosomal Segment Substitution Lines (CSSLs) derived from an *Indica* nitrogen deficiency tolerant Xieqingzao B (XQZB) and an *Indica* nitrogen deficiency susceptible Zhonghui9308 (ZH9308) was studied. A genetic linkage map was constructed by employing 87 SSR and 33 In Del markers to analyze quantitative trait loci (QTLs) at seedling stage in rice under normal (NN), low (LN) and relative nitrogen (LN/NN) levels. A sum of 9 QTLs was detected in 6 regions on 6 chromosomes, with each QTL explaining 10.58%-27.27% of phenotypic variation. The QTLs controlling shoot dry weight and total dry weight were simultaneously located at RM24718 interval on chromosome 9 under low N conditions. QTLs responsible for root dry weight under LN situations were detected near the marker InD90 on chromosome 6, which explained 16.84% of the phenotypic variation and had a high value for its additive effect (6.14). For future genetic improvement programs, the root dry weight attribute is considered distinctly, which helps to improve, N-deficiency tolerance. This study also providesinsights into fine mapping, gene cloning, and marker assisted selection for future research.

Key words: Nitrogen, Quantitative trait loci, Rice, Root traits.

Introduction

Rice (*Oryza sativa* L.) is an important source of protein and calories for almost half of global population (Rohit & Parmar, 2011). Fertilizers, mainly Nitrogen (N), potassium (K) and Phosphorous (P) are considered basic components for normal plant growth and production. Meanwhile, nitrogen use efficiency (NUE) reduces with an increase in N fertilizer application (Noor, 2017). Whereas, nitrogen fertilizer consumption in China accounts for one third of the global total, with very low NUE approximately 30% (Leo, 2016; Peng *et al.*, 2002). Due to inappropriate application methods and time of N fertilizer along with less NUE, the major portion of N is unable to be used efficiently because of its volatile nature and leaching, posing indirect problems to human health and environmental pollution (Peng *et al.*, 2006; Xing & Zhu, 2000). Hence, development of germplasm having high NUE is among the key objectives of any crop breeding programs (Zhao *et al*., 2014). Nitrogen significantly improves the rice grain yield by enhancing other yield related attributes e.g. panicle number, thousand grain weight and spikelet fertility (Fageria, 2007). Additionally, nitrogen deficit leads toward a reduction in plant growth which ultimately causes yield reduction (Zhou *et al*., 2018). For, sustainable rice production tolerance to low nitrogen stress is an important attribute.

Roots are integral components for different functions, like water uptake and nutrient efficiency, physiological function, anchoring and mechanical support which, are the major interface among the plants and various other biotic and abiotic stresses in the soil's environment (Smith & Smet, 2012). Root's response to nitrogen deficiency seems to be highly reliant on prevailing environmental factors, plant's species and individual variations among cereal crops (Hermans *et al.,* 2006). Analyzing root attributes, like its length, total number, root and shoot dry weight and total dry weight are important characters in rice breeding programs (Kanbar *et al.*, 2009).

The phenotypic variations which are due to quantitative genes can differ depending on various allelic combinations which possibly lead toward continuous variation in major traits. Quantitative trait loci (QTL) mapping is employed to study these multigenic attributes. QTL analysis had the advantage that many genetic components and interaction among the loci can be analyzed at the same time. This is of specific interest for growth-related components, which are mainly caused by many genes (Rebouillat *et al.*, 2009; E *et al.*, 2012). QTLs can be expressed under one condition while changes effect when conditions differ. Variations caused by nature holds the potential to identify a genetic material having better nutrient uptake capability and utilization efficiency, and QTL analyses by utilizing natural variation have already identified genes involved in nutrient deficiency (Rengel & Marschner, 2005). In another study, some QTLs were mapped from recombinant inbred line (RIL) population of 'Zhenshan 97'/'Minghui 63' at seedling stage for LN tolerance and noticed locations of few QTLs parallel to the locus of genes responsible to N assembling and its allocation e.g. NADH-GOGAT, GS and GDH2 (Lian *et al.*, 2005). QTLs control root morphological and physiological characteristics. The root architecture system commonly consists of root length, weight, total number, thickness and density of primary, adventitious and lateral roots (Obara *et al.*, 2010). Whereas, a root architecture system study is complicated to tackle in soil conditions.

To avoid these restrictions, a number of studies have been conducted for investigating root architecture by growing rice seedlings under hydroponic condition through diverse mapping populations (Obara *et al.*, 2010; Ogawa *et al.*, 2014). It has been observed that QTLs for root length identified from hydroponic studies may be helpful for elevating grain yield in fields (Obara *et al.*, 2011). A similar approach was employed broadly for the detection of QTLs responsible for improved root architecture under normal and stressed field's condition (Shimizu *et al.*, 2004). Therefore, the present research was designed to analyze the genetic basis for root architecture and its development in rice at the seedlingstage and to detect desirable genes for root traits.

Materials and Methods

Plant materials: The population comprised of 75 CSSLs utilized and were screened at the early seedling stage by employing N-deficiency tolerance trials. The mapping population (75 CSSLs) was derived from a cross between an *Indica* restorer line, Zhonghui 9308 (susceptible to N deficiency), and an *Indica*maintainer line, Xieqingzao B (tolerant to N deficiency), parents of Xieyou 9308, a super hybrid rice in China (Fig. 1). The parents were planted as controls. The CSSL populations were developed as follows: the F_1 hybrid (Xieqingzao B × Zhonghui 9308) was continuously inbred for thirteen generations; it was then crossed with the recurrent parent Zhonghui 9308; thereafter, in each following generation, plants with characteristics differences from the recurrent parent were selected and separately backcrossed with the recurrent parent to establish the CSSLs. The backcross was continued for four generations, and inbreeding was continued for six generations. A total of 75 CSSL lines were selected and used for further phenotypic evaluation (Fig. 1).

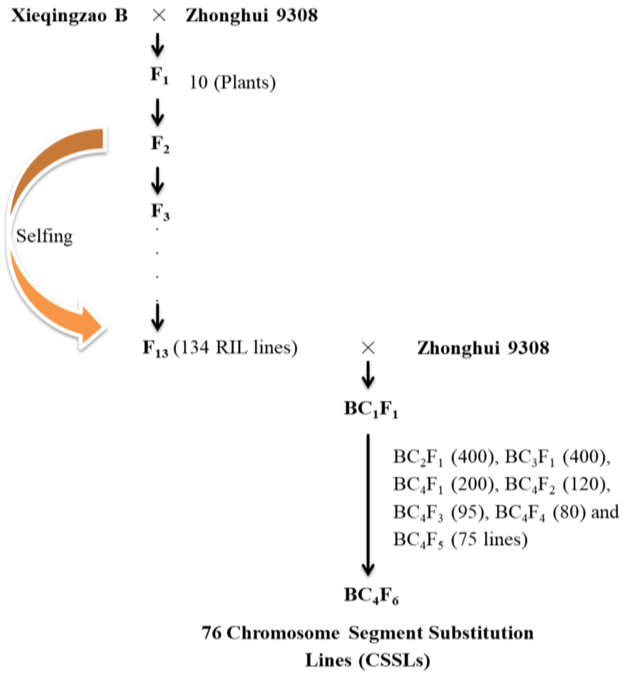

Fig. 1. Breeding scheme demonstrating the development of chromosome segment substitution lines (CSSL) populations.

Hydroponic culture experiment: The present investigation was carried outat the China National Rice Research Institute (CNRRI), Hangzhou, China during 2016 and 2017. The experiment was conducted in a glasshouse by maintaining a day/night temperature of 30/21°C, with relative humidity at 70% and natural daylight. The CSSLs and their parents were raised under hydroponic conditions. Seeds for each CSSL and their parents were soaked in tap water at 37°C for 48 hours. Afterward, thirteen germinated seeds were plugged on a floating foamed plastic using pots in two pools, each 6.0 m long, 1.1 m wide and 0.5 m deep. One pool had the optimum nitrogen concentration while the other one was with low nitrogen concentration. The nutrient solution in the normal nitrogen pool was the mixture of (mg/l); 48.2 $(NH_4)_2SO_4$, 59.9 $Ca(NO_3)_2$, 24.8 KH_2PO_4, 18.5 KNO_3, 15.9 K_2SO_4, and 65.9 $MgSO_4$ and 1 ml/l Fe-EDTA, H_3BO_3, $CuSO_4{\cdot}5H_2O$, $ZnSO_4{\cdot}7H2O$, $MnCl_2{\cdot}4H_2O$, and $H_2MoO_4{\cdot}H_2O$. The extent of low N tolerance was considered as reported by Lian *et al.* (2005). The experiment was comprised of three set of replicates, with 10 seedlings per each replication. Whereas, inter-row and -plant distance kept at 3.0 x 3.0 cm. The mixed nutrient solution was filled in the ponds at the time of transplanting and was refreshed every 10 days.

Phenotyping for studied traits in CSSLs: For phenotypic investigation, the 75 CSSLs and the two parental lines were grown at a hydroponic experiment to study six traits including plant height (PH), root length (RL), root number (RN), shoot dry weight (SDW), root dry weight (RDW) and total dry weight (TDW). Ten plants were sampled and measured from each plot under two N levels at the seedling stage for these phenotypic traits. After measuring the root length, the roots were cut off, placed in an envelope and dried in an oven at 70°C. Correlation coefficient for all the studied traits were computed using SPSS Statistical package version 17.0 (SPSSInc., Chicago, IL, USA). The nitrogen levels were marked NN, LN and LN/NN for normal N level, low N level, and their relative N ratio, respectively.

QTL mapping: In this study, we constructed a series of 75 Chromosomal Segment Substitution Lines from backcross progeny (BC_4F_6) derived from a cross between Zhonghui 9308 as the recurrent parent and Xieqingzao B as the donor parent. In this process, we carried out 87 SSR and 33 InDel markers, distributed along the rice genome in accordance with already described linkage maps, and similarly known polymorphisms among parents were employed in identifying the genotypes of the CSSL population. The chromosomal locations of putative QTLs were located via inclusive composite interval mapping with QTL IciMapping 4.0 (Wang, 2009). A locus showing a threshold LOD value > 2.00 and $p<0.05$ was declared to be a putative QTL. Whereas, contribution rate was calculated as the percentage variance explained by each locus or epistatic pair in proportion to the total phenotypic variance. Standard conventions of nomenclature were adopted in naming QTLs (McCouch, 2008).

Table 1. Phenotypic analysis of the Chromosomal Segment Substitution Lines (CSSLs) population and the parents under NN and LN levels.

Level	Traits	Parents		CSSLs				
		Xieqingzao B	Zhonghui 9308	Mean	Range	Kurtosis	Skewness	SD %
NN	PH	20.68	21.67	21.06	16.52-27.82	0.79	0.12	0.02
	RL	7.54	6.34	6.56	4.43-9.23	-0.58	0.20	1.14
	RN	6.50	6.00	6.18	5.00-8.67	-0.19	0.47	0.80
	SDW	84.33	79.00	80.91	55.38-104.83	-0.25	0.27	10.64
	RDW	32.67	27.67	26.59	18.33-39.0	-0.46	0.64	5.13
	TDW	117.00	106.67	107.51	82.01-142.13	-0.47	0.48	14.65
LN	PH	18.30	18.87	18.65	14.29-24.20	-0.26	0.15	2.16
	RL	8.63	7.83	7.76	5.45-10.38	-0.18	0.01	1.06
	RN	9.35	7.83	7.15	5.71-9.25	-0.37	0.13	0.73
	SDW	86.17	72.33	77.06	50.29-98.88	-0.16	-0.15	10.70
	RDW	64.04	44.72	34.20	21.33-46.33	0.07	0.09	4.85
	TDW	125.21	117.06	111.27	79.29-143.33	-0.28	-0.03	13.94

NN, normal nitrogen; **LN**, low nitrogen; **PH**, plant height; **RL,** root length; **RN**, root number; **SDW**, shoot dry weight; **RDW**, root dry weight; **TDW**, total dry weight.

Table 2. Correlation coefficients among six root traits under two N levels in the CSSL populations.

Traits	PH	RL	RN	SDW	RDW	TDW
PH	***0.00***	0.324*	0.399**	0.606**	0.364*	0.567**
RL	0.564**	***0.00***	0.442**	0.612**	0.647**	0.671**
RN	0.320*	0.182	***0.00***	0.577**	0.616**	0.635**
SDW	0.552**	0.397*	0.407**	***0.00***	0.689**	0.967**
RDW	0.273*	0.277*	0.148	0.542**	***0.00***	0.851**
TDW	0.518**	0.401**	0.364*	0.956**	0.764**	***0.00***

The data show the correlations between the six traits under two nitrogen (N) levels. The data above the diagonal correspond to correlations with normal nitrogen (NN) and below correspond to correlations with low nitrogen (LN); **PH**, plant height; **RL,** root length; **RN**, root number; **SDW**, shoot dry weight; **RDW**, root dry weight; **TDW**, total dry weight; and * and ** for significance at 0.05 and 0.01 probability levels, respectively

Results

Parents and CSSL population performance: A total of 75 CSSLs and their parents were investigated during the whole developmental period. For most of the plants in fields, they grow rapidly and their phenotypes were more similar to the recurrent parent Zhonghui 9308. The phenotypic data of the six traits, including PH, RL, RN, SDW, RDW and TDW in the parents and CSSL populations under two N levels (normal and low), are listed in (Table 1). As shown in Table 1, the measured traits of RL, RN, SDW, RDW and TDW in Zhonghui9308 were less than those in XieqingzaoB under two N levels, thereby suggesting that Zhonghui 9308 less responsive to N deficiency than Xieqingzao B (Fig. 2). On the other hand, plant height for Zhonghui9308 was greater than those in Xieqingzao B under two N levels. The phenotypic values of CSSL populations with normal N levels were higher than those with low N levels, especially for PH and SDW. PH under low N was 2.41 g less than PH under normal N with the decreased rate of 12.92%, and SDW was 3.85 g with the decreased rate of 4.75%. The remaining studied traits (RL, RN, RDW, and TDW) for CSSLs under low N were higher than those under normal N. This significant difference of the phenotypic values of the four traits between the two N levels indicates the possibility of developing new lines tolerant to N deficiency. Histograms showing the distribution of the phenotypic variation for the six traits in the 75 CSSL populations are presented in Fig. 3. The phenotypic variation of the six traits displayed a continuous distribution, and the skew values ranged from -0.03 to 0.64 (<1, suggesting near normal distributions). It also illustrated that the frequency distribution of the six traits varied widely, and there existed transgressive segregations, indicating polygenic inheritance.

Phenotypic correlations among the traits: The phenotypic correlation coefficients during the seedling stage under two N levels among the six studied traits are highlighted in (Table 2). Data below the diagonal correspond to correlations with LN levels and data above the diagonal correspond to correlations with NN levels. Moreover, the strongest correlations were found between SDW and TDW under both N levels, 0.967** under the NN level, and 0.956** under the LN level. Of all the combinations of the six traits, thirteen pairs showed significant positive correlations with each other. TDW was positively correlated with RL, SDW, and RDW under both N levels, indicating that root development is very important for plant architecture under low N stress. In addition, there were no significant correlations between RN and RL with RDW under LN level conditions.

QTL analysis for attributes under NN, LN and LN/NN levels: Total, 9 QTLs identified under two nitrogen (N) levels, LOD values ranging from 2.14 to 5.18 which, explained the phenotypic variance ranging from 10.58% to 27.27%. One QTL detected under normal nitrogen (NN) conditions, 3 under low nitrogen (LN) and 5 with respect to the LN/NN ratio; the QTLs were distributed on the rice chromosomes 5, 6, 7, 8, 9 and 11 (Table 3 and Fig. 4).

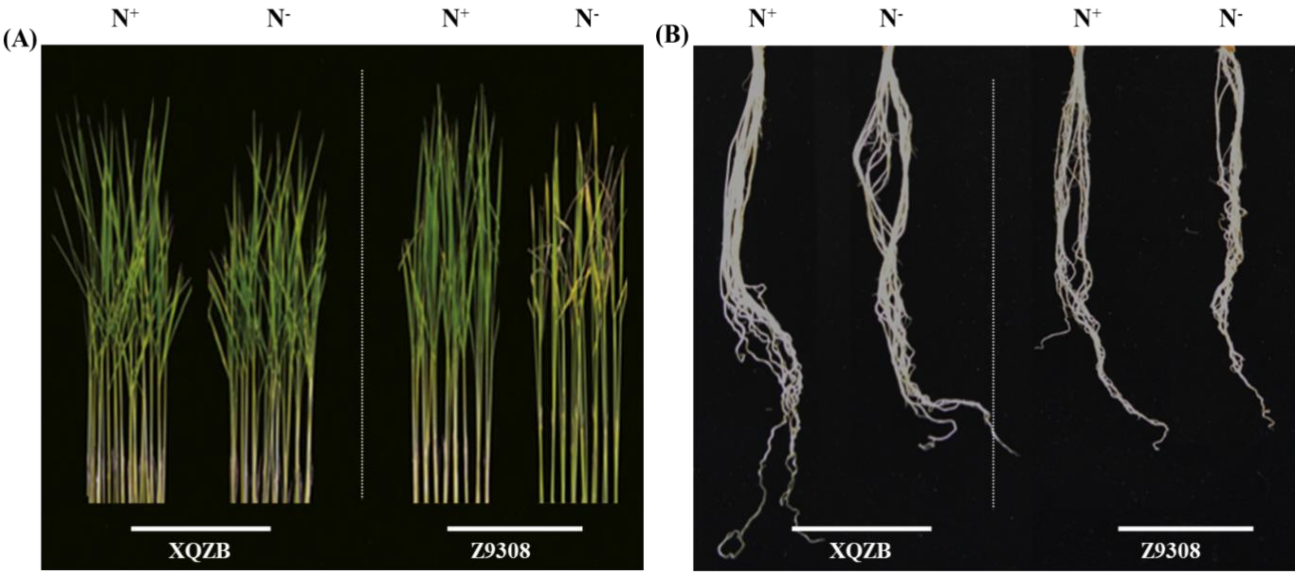

Fig. 2. Phenotype of the rice parental lines Xieqingzao B and Zhonghui 9308 under two Nitrogen N+ and N- levels. (a, b) Images shoot and root of the two parents after four weeks under hydroponic conditions, N+ normal conditions; N- deficient conditions.

Three putative QTLs (*qPH-6, qPH-8,* and *qPH-11*) for PH were identified from the NN level and LN/NN ratio. Under the normal N level, only one QTL was mapped to chromosome 6, which explained 16.94% of the total phenotypic variation. Two QTLs (*qPH-8* and *qPH-11*) from the LN/NN ratio analysis were found on chromosomes 8 and 11; these individual QTLs explained 10.58% and 20.68% of the total phenotypic variation, respectively. One QTL (*qRL-7*) responsible for root length was identified on chromosome 7 from the LN/NN ratio analysis. The total phenotypic variation of about 12.33% was explained by QTL, and the allele *qRL-7* was inherited from the XQZB parent with an additive effect of 0.18. One QTL (*qRN-5)* for root number was detected on chromosome 5 from the LN/NN ratio and 12.74% of the total phenotypic variation was explained in this way. This QTL came from the XQZB parent with an additive value of 0.13.Two QTLs for shoot dry weight were identified from the LN level and LN/NN ratio. The QTLs *qSDW-9* and *qSDW-11* were found located on chromosomes 9 and 11, and phenotypic variations of 13.13% and 27.27% were explained by every QTL, respectively; collectively, they explained 40.40% of the total phenotypic variation. The QTL for shoot dry weight *qSDW-11* was detected in the same region as in *qPH-11* on chromosome 11 (Fig. 4). The additive effect for *qSDW-11* was positive and *qSDW-9* was negative, with values of 0.41 and -9.83, respectively. From the low N level, one QTL (*qRDW-6*) was detected on chromosome 6 with an additive effect value of 6.14, and the phenotypic variations explained and the LOD values were 16.84% and 3.0, respectively. The QTL for root dry weight *qRDW-6* was detected in the same region as in *qPH-6* on chromosome 6 (Fig. 4). The positive allele for QTL (*qRDW-6*) was from the large root dry weight parent XQZB. For the total dry weight trait, one QTL (*qTDW-9*) from the low N level was identified on chromosome 9, which explained 13.01% of total phenotypic variation showed the negative additive effect of -12.74. The allele *qTDW-9* was inherited from the parent ZH9308 and decreased the total dry weight.

Table 3. Putative quantitative trait loci (QTL) detected for root traits under low N, normal N and LN/NN levels in a CSSLs population derived from XQZB and ZH9308.

N Level	Trait	Progeny QTL	Chr.	Marker name	LOD	PVE%	Add
NN	PH	*qPH-6*	6	InD90	3.02	16.94	2.56
LN	SDW	*qSDW-9*	9	RM24718	2.29	13.13	-9.83
	RDW	*qRDW-6*	6	InD90	3.00	16.84	6.14
	TDW	*qTDW-9*	9	RM24718	2.27	13.01	-12.74
LN/HN	PH	*qPH-8*	8	InD113	2.33	10.58	0.06
	PH	*qPH-11*	11	RM26652	3.77	20.68	0.18
	RL	*qRL-7*	7	RM5436	2.14	12.33	0.18
	RN	*qRN-5*	5	RM3638	2.22	12.74	0.13
	SDW	*qSDW11*	11	RM26652	5.18	27.27	0.41

Add, additive effect of QTLs; PVE, phenotypic variance of QTLs; LOD, logarithm of odds; qPH, QTL for plant height; qRL, QTL for root length; qRN, QTL for root number; qSDW, QTL for shoot dry weight; qRDW, QTL for root dry weight and qTDW, QTL for total dry weight

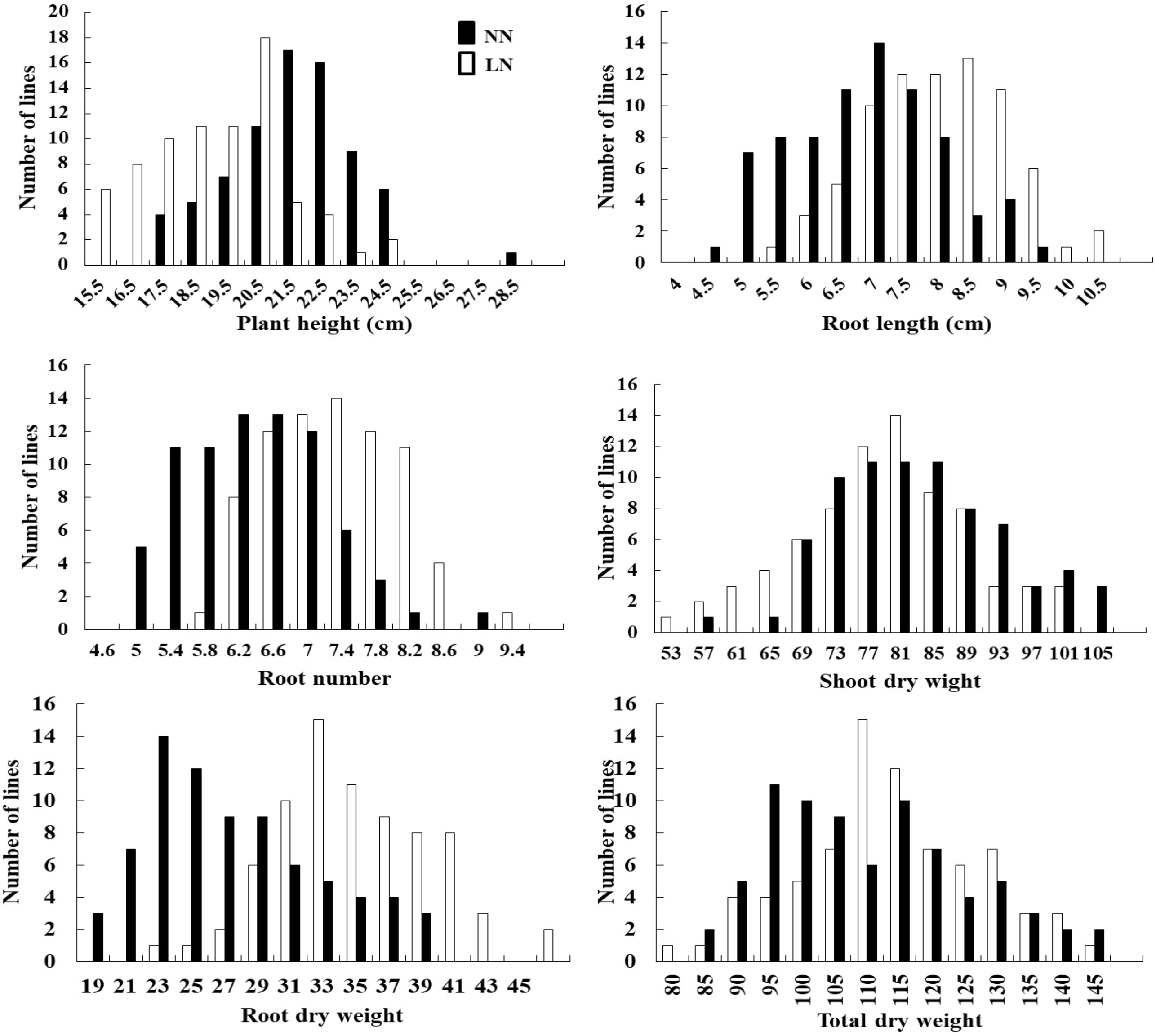

Fig. 3. Frequency distribution of plant height, root length, root number, shoot dry weight, root dry weight and total dry weight of 75 CSSL populations under low nitrogen (LN) and normal nitrogen (NN) levels.

Discussion

Nitrogen (N) being an integral nutrient for plant growth and development, plays a pivotal role in regulating rice crop yield (Su *et al.,* 2005). However, intensified N fertilizer application may cause an impairment in rice quality, environmental hazards and other associated problems. Thus, approaches for hampering rice tolerance for low N have become a research focus. Specifically, numerous researches have declared major genotypic variances in rice based on N deficiency tolerance (Jiang *et al.,* 2003; Piao *et al.,* 2003; Cao *et al.,* 2006; Cheng *et al.,* 2012; Feng *et al,* 2015). Genotype × environment interaction is among the several aspects influencing the expression of multi genic characters (Fan *et al*., 2005). Root attributes are also multi genic and are environment dependent (Cho *et al.,* 2007). Feng*et al.* (2010) analyzed the QTLs for root traits of rice under different N levels and identified seven QTLs on chromosomes 1, 2, 3 and 8 linked with N-deficiency tolerance, thus confirming the possible interactions between QTLs for roots traits and different N levels. Our results were in line with aforementioned research, in terms of demonstrating possible interactions among detected QTLs and N levels. Such interactions revealed the differential expression of genes under variable N situations. Zhao *et al.* (2015) detected 28 QTLs under two N levels and 16 QTLs of their relative traits. In present study aimed at identifying and mapping of QTLs associated with root traits, we assessed 75 CSSLs derived from a cross between XQZB and ZH9308 under two N levels and their relative traits. The root traits under the LN level lowered in lesser quantities in Zhonghui 9308 as compared to Xieqingzao B, thereby designating Zhonghui 9308 as stronger N-deficiency tolerant compared to Xieqingzao B. The results were consistent with previous studies (Feng *et al.,* 2010). Nine QTLs, including one QTL under normal nitrogen (NN), 3 under low nitrogen (LN) and 5 from the LN/NN ratio, were mapped. Several QTLs for the plant height of rice have been detected (Feng *et al.,* 2010; Yong-shu *et al.,* 2011). A total of three QTLs influencing plant height were detected under variable N levels (NN and LN/NN ratio) in this study. Of these, the QTL of *qPH-6* had the largest additive effect from the NN level, contributing 16.94% of the observed phenotypic variation, the

Xieqingzao B allele at the QTL caused an increase in plant height. In addition, one QTL affecting plant height was mapped on chromosome 6 from the NN level in this study, and these results are in line with Zhao *et al.* (2015) and Lin *et al.* (2011). Additionally, Xu *et al.* (2004) identified one QTL for seedling height in the vicinity region of *qPh2b* by using a different population at the seedling stage, indicating there are different alleles related to rice plant height. One QTL for root length on chromosome 7 was only detected from the LN/NN ratio, individually contributing 12.33% of the total phenotypic variation. The allele for this QTL was inherited from Xieqingzao B with a positive trait value. This shows that this genomic region might be associated with rice resistant to low-N stress. Moreover, Xing *et al.* (2002) detected a QTL conferring root length of rice in the adjacent region, indicating that a long root length might enhance the capability of rice plants to absorb and use nitrogen, thus influencing the plant height. In the present study, QTLs for shoot dry weight and total dry weight were simultaneously associated with the marker RM24718 on chromosome 9 under the low N level, showing that this genomic region might be associated with rice resistant to low-N stress, which was also located in the genomic region reported by Wei *et al.* (2012) and Zhao *et al.* (2015). In the present study, one of the QTLs (*qRDW-6*) related to root dry weight in both N levels (NN and LN) explained 16.84% phenotypic variation on chromosome 6, which is associated with the marker InD90. The allele *qRDW-6* was derived from the low-N tolerant parent XQZB and had a positive additive effect of 6.14.

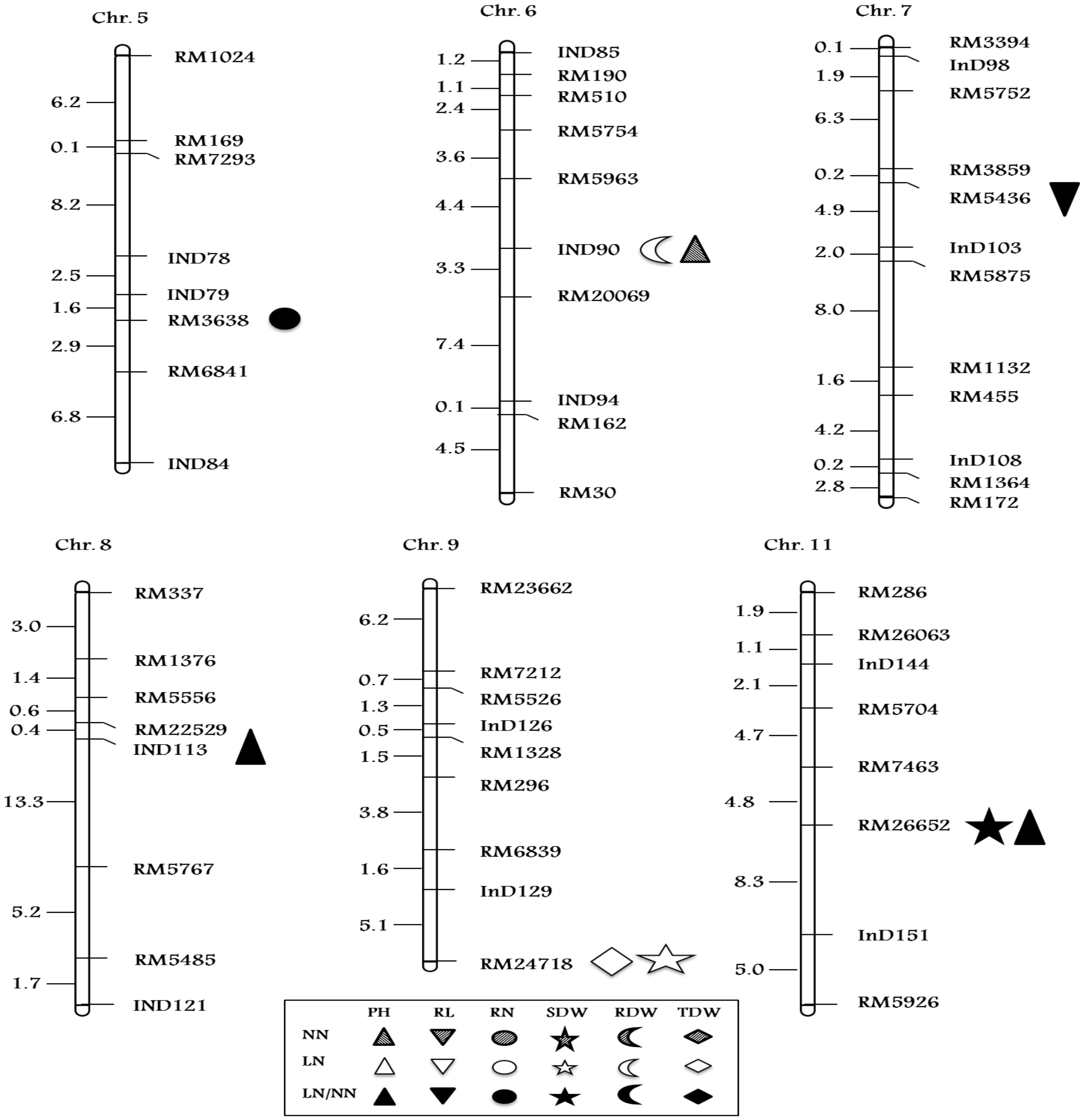

Fig. 4. Location of QTLs for rice root attributes under LN, NN, and LN/NN levels.PH, plant height; RL, root length; RN, root number; SDW, shoot dry weight; RDW, root dry weight; TDW, total dry weight.

Conclusion

The current study aimed at characterizing the genetic basis of root architecture and its development in rice at the seedlingstage in different N levels will be helpful in categorizing rice lines having superior root architecture and development system and will assist in breeding rice germplasm with low-N tolerance by molecular assisted markers. One of the CSSLs (CL-45) would be suitable to select for further study on the basis of the genetic map (unpublished) of the 75 chromosome segment substitution lines. In future genetic improvement evaluations, the root dry weight could be considered as the primary focus for achieving increased root dry weight towards the breeding of N-deficiency tolerant lines. Fulfilling the said objectives, present work provides the useful information for fine mapping, gene cloning, and marker assisted selection for desirable rice root traits in future research.

Acknowledgements

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